NATIONAL STANDARD OF THE PEOPLE'S REPUBLIC OF CHINA

GB 5009.247-2025

Replacing GB 5009.247-2016

National food safety standard - Determination of neotame in foods

食品安全国家标准 食品中纽甜的测定

Issued on: March 16, 2025 **Implemented on: September 16, 2025**

Issued by: State Administration for Market Regulation;
Standardization Administration of the PRC

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2 Principle (first method)

The test portion is extracted with a mixed extraction solution; samples containing a gum base are dissolved in a water bath, high-fat samples are defatted with n-hexane, protein is precipitated with a protein precipitant and the extract is cleaned up by solid phase extraction before measurement on a liquid chromatograph, with identification by retention time and quantification by external standard.

3 Reagents and materials (first method)

Unless otherwise stated the reagents are of analytical grade and the water is grade one water as specified in GB/T 6682. The reagents listed are methanol, formic acid, ammonium acetate and glacial acetic acid, all of chromatographic grade, together with triethylamine,

potassium ferrocyanide trihydrate, zinc acetate dihydrate, ammonia solution and n-hexane.

3.2 The prepared solutions are as follows. The formic acid-triethylamine buffer is made by taking 0.8 mL of formic acid and 2.5 mL of triethylamine, making up to 1 L with water, giving a pH of about 4.5. The mixed extraction solution is made by measuring 400 mL of methanol into a 1 L beaker, adding 500 mL of the formic acid-triethylamine buffer, adjusting the pH to 4.5 with formic acid, transferring to a 1 L volumetric flask and making up to the mark with the buffer. The mixed eluting solution is made by measuring 700 mL of methanol into a 1 L volumetric flask and making up to the mark with the buffer. The 20 mmol/L ammonium acetate solution is made by weighing 1.54 g of ammonium acetate, dissolving it in 500 mL of water, adding 1.0 mL of glacial acetic acid and making up to 1 L with water. The potassium ferrocyanide solution, labelled 92 g/L, is made by weighing 106 g of potassium ferrocyanide, dissolving it in a suitable volume of water and making up to 1 L. The zinc acetate solution, labelled 183 g/L, is made by weighing 220 g of zinc acetate, dissolving it in a suitable volume of water, adding 30 mL of glacial acetic acid and making up to 1 L with water. The 20 percent ammonia solution is made by measuring 200 mL of ammonia solution into a 1 L volumetric flask and making up to the mark with water.

3.3 to 3.4 The standard substance is neotame, molecular formula C20H30N2O5, CAS number 165450-17-9, of purity not less than 99.0 percent, or a certified reference material issued with a national certificate. The standard stock solution at 1.00 mg/mL is made by accurately weighing 100 mg of the standard substance, to the nearest 0.0001 g, dissolving it in the formic acid-triethylamine buffer and making up to 100 mL; it is kept at 4 °C in the dark and is valid for 3 months. Working standard solutions at 0.500 μg/mL, 1.00 μg/mL, 5.00 μg/mL, 10.0 μg/mL, 50.0 μg/mL and 100 μg/mL are prepared from the stock solution with the same buffer and made up freshly before use.

3.5 The materials are a solid phase extraction cartridge of 500 mg per 6 mL packed with polystyrene pyrrolidone polymer, or one of equivalent performance, and an organic microporous membrane filter of 0.45 μm.

4 Apparatus and equipment (first method)

The apparatus comprises a high performance liquid chromatograph with an ultraviolet or diode array detector; balances with readabilities of 0.01 g and 0.0001 g; a pH meter accurate to 0.01; a vortex mixer; a centrifuge with a speed of at least 4000 r/min; a nitrogen evaporator; an ultrasonic cleaner; a grinder; a water bath; and a solid phase extraction manifold.

5 Analytical procedure (first method)

5.1.1 Liquid samples are shaken and held for extraction; semi-solid samples of uniform matrix and powdered samples are held for extraction as they are; other samples are

homogenised or ground until uniform. The prepared test portions are kept at 0 °C to 5 °C until analysis.

5.1.2 For sweets containing a gum base, jellies, chicken essence seasoning and similar samples, 5 g of the test portion is accurately weighed to the nearest 0.01 g into a 50 mL centrifuge tube, 25 mL of the mixed extraction solution is added and the tube is shaken in a water bath at 60 °C for 30 min. After cooling to room temperature the pH is adjusted to 4.5 with formic acid or the 20 percent ammonia solution and the tube is sonicated for 30 min. Then 1 mL of the potassium ferrocyanide solution and 1 mL of the zinc acetate solution are added, the tube is vortexed and centrifuged at 4000 r/min for 5 min, and the supernatant is filtered through paper into a 50 mL volumetric flask. The residue is extracted again with 20 mL of the mixed extraction solution, shaken for 5 min and centrifuged at 4000 r/min for 5 min, the supernatant is filtered and combined, and the flask is made up to the mark with the mixed extraction solution to give the sample extract for clean-up. For fat-based seasonings, chocolate, cream and similar samples, the same 5 g portion is extracted with 25 mL of the mixed extraction solution in a water bath at 60 °C for 30 min, 5 mL of n-hexane is added, the tube is shaken for 5 min and centrifuged at 4000 r/min for 5 min, the hexane layer is discarded and the procedure continues from the pH adjustment step. For all other samples the 5 g portion is vortexed with 25 mL of the mixed extraction solution for 5 min and the procedure continues from the pH adjustment step.

5.1.3 The solid phase extraction cartridge is conditioned before use with 6 mL of methanol followed by 6 mL of water and kept wet. 10.0 mL of the sample extract is passed through the cartridge, which is washed with 5 mL of the formic acid-triethylamine buffer, all the effluent being discarded. The analyte is eluted with 8 mL of the mixed eluting solution, the eluate is concentrated under nitrogen in a water bath at 60 °C to about 1 mL and made up to 2.0 mL with the formic acid-triethylamine buffer to give the test solution.

5.2 The reference chromatographic conditions are a C18 column of 150 mm x 4.6 mm packed with 5 μm material or an equivalent; a column temperature of 30 °C; mobile phase A methanol and mobile phase B the 20 mmol/L ammonium acetate solution, with the gradient of Table 1; a flow rate of 1.0 mL/min; a detection wavelength of 210 nm; and an injection volume of 50 μL. Table 1 gives the gradient in three columns, time in minutes and the percentages of mobile phases A and B: at 0.00 min, 10 percent A and 90 percent B; at 4.00 min, 60 percent and 40 percent; at 20.00 min, 60 percent and 40 percent; at 20.01 min, 10 percent and 90 percent; and at 25.00 min, 10 percent and 90 percent.

5.3 to 5.5 The working standard solutions are injected in turn and the corresponding peak areas measured; the calibration curve is drawn with the neotame concentration of the working standard solutions on the horizontal axis and the peak area response on the vertical axis, and the chromatogram of the standard solution is shown in Figure A.1 of Annex A. The test solution is filtered through a 0.45 μm membrane and injected, neotame being identified by retention time and quantified by peak area, its concentration in the test

solution being read from the calibration curve. A blank test is carried out by following the whole procedure without the test portion.

6 Expression of results (first method)

The neotame content of the test portion is calculated by Formula (1), whose symbols are: X, the neotame content of the test portion, in milligrams per kilogram; rho, the mass concentration of neotame in the test solution taken from the calibration curve, in micrograms per millilitre; V, the volume to which the test solution was made up, in millilitres; V1, the volume to which the sample extract was made up, in millilitres; 1000, the conversion factor; m, the mass of the test portion, in grams; and V2, the volume of sample extract used for clean-up, in millilitres. The result is reported to three significant figures.

7 Precision (first method)

The absolute difference between two independent results obtained under repeatability conditions shall not exceed 15 percent of their arithmetic mean.

8 Other (first method)

For a test portion of 5 g the detection limit of the method is 1 mg/kg and the quantification limit is 5 mg/kg.

9 Principle (second method)

The test portion is extracted with the mixed extraction solution; samples containing a gum base are dissolved in a water bath, high-fat samples are defatted with n-hexane, protein is precipitated with a protein precipitant and the extract is cleaned up by solid phase extraction before measurement on a liquid chromatograph-tandem mass spectrometer, with identification by retention time and by the abundance ratio of the characteristic ions, and quantification by external standard against a matrix-matched calibration curve.

10 Reagents and materials (second method)

The reagents and the water are as for the first method, and the reagents listed are the same. The only additional preparation is a 5 mmol/L ammonium acetate solution, made by weighing 0.385 g of ammonium acetate, dissolving it in 500 mL of water, adding 1.0 mL of glacial acetic acid and making up to 1 L with water; the other solutions are those of 3.2 and the standard substance is that of 3.3.

10.4 The standard stock solution at 1.00 mg/mL is prepared as in the first method, by accurately weighing 100 mg of the standard substance to the nearest 0.0001 g, dissolving it in the formic acid-triethylamine buffer and making up to 100 mL, kept at 4 °C in the dark and valid for 3 months. An intermediate standard solution at 10.0 µg/mL is prepared from it